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Glomus crenatum (*Glomeromycetes*), a new ornamented species from Cuba

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Abstract — A new ornamented species of the *Glomeromycetes* found in Pinar del Rio and Moa, western and east Cuba, respectively, is proposed here as *Glomus crenatum*. The fungus differs from previously described species by the darkly pigmented spore walls that possess a hemispherical dome-like surface ornamentation.

Key words — Caribbean, *Glomeromycota*, *Glomerales,* tropical forest

Introduction

Among several recently described species of the genus *Glomus* (Błaszkowski et al. 2009a,b, 2010a,b; Cano et al. 2009; Furrazola et al. 2010) only few produce ornamented spores (Błaszkowski et al. 2004, Hu 2002, Oehl et al. 2003). In South and Central America few species with glomoid glomerospores have been described, such as *Glomus brohultii* Sieverd. & R.A. Herrera 2003, *G. patagonicum* [≡ *Pacispora patagonica* (Novas & Francchia) C. Walker et al. 2007], *P. boliviana* Sieverd. & Oehl 2004, *G. megalocarpum* D. Redecker 2007, and a glomoid synanamorph of *Ambispora brasiliensis* B.T. Goto et al. 2008 (Herrera-Peraza et al. 2003, Oehl & Sieverding 2004, Novas et al. 2005, Walker et al. 2007, Redecker et al. 2007, Goto et al. 2008). Only *Pacispora patagonica* and *P. boliviana* have an ornamented outer spore wall but these species differentiate anew an inner wall during spore development and a germinal orb specialized to produce germinal tube initiation (Oehl & Sieverding 2004, Walker et al. 2004).

Cuba has a high diversity of soils, many endemic plants, and different ecosystems that probably harbour many undescribed microorganisms, including arbuscular mycorrhizal fungi (AMF). The native soils of the Cuban biosphere reserve "Sierra del Rosario" have been surveyed for arbuscular mycorrhizal (AM) fungi within a long-term ecological and biodiversity study (Herrera et al. 1988, Herrera-Peraza et al. 1994, Ferrer & Herrera 1988). One glomerospore found during this work is described herein as *Glomus crenatum*.

Material & methods

Site ecology

The UNESCO Biosphere Reserve "Sierra del Rosario" is located at the eastern section of the "Sierra del Rosario" mountains (22°45′ to 23°00′N, 82°50′ to 83°10′W) extending over both provinces of Pinar del Rio and La Habana. The mountain range reaches altitudes up to 565 m. The 20-year annual temperature and rainfall averages at the reserve are 24.4°C and 2014 mm (Herrera et al. 1988). Ten soil samples were collected in native evergreen mesophyllous forests or secondary forests dominated by *Syzygium jambos* (L.) Alston (locally called "Pomarrozal"), an exotic, invasive tree species. The native evergreen mesophyllous forest community is commonly dominated by *Pseudolmedia spuria* (Sw.) Griseb., *Oxandra lanceolata* (Sw.) Baill., *Trophis racemosa* (L.) Urb., *Matayba apetala* (Macfad.) Radlk., *Dendropanax arboreus* (L.) Decne. & Planch. and *Calophyllum antillanum* Britton, although the composition varies with topography. Trees heights are commonly 10–15 m on exposed, sunny hilltops, 20–25 m on lower slopes, and ≤ 35–40 m in deep protected "V" valleys where nutrient and water usually is available all year. *Syzygium jambos,* an opportunistic invasive species that quickly colonizes cut areas, can also colonize pure stands, being then very difficult to eradicate. Another sample was collected in Moa, Northeastern Cuba, from a plantation of *Pinus cubensis* Griseb. saplings shorter than 2 m, where AM hosts, such as *Cecropia* spp. and other indigenous plants, were also present.

Morphological analyses

Spores were separated from soil samples by wet-sieving and decanting (Gerdemann & Nicolson 1963) followed by gradient centrifugation in 1M sucrose (Sieverding 1991). Glomerospores were then placed in dishes with water and separated under a dissecting microscope. Spores were mounted on microscope slides either in water (to check unmodified spore wall components; Spain 1990) or permanently in polyvinyl-lacto-glycerol (PVLG; Omar et al. 1979) or PVLG with Melzer's reagent. Permanent slides were also prepared using Farrant's (Arabic gum, 40 g; water, 40 ml; glycerol, 20 ml; phenol, 20 mg) and Hoyer's (arabic gum, 20 g; water, 25 ml; glycerol, 10 ml; chloral hydrate, 100 g) mounting fluids. Spores were also examined in PVLG-Cotton Blue 0.05 % to assess the reaction of the spore wall components.

Terminology for the species description was adopted from Oehl et al. (2003) and Goto & Maia (2006). Zeiss Axioskop compound microscopes with or without Nomarski differential interference contrast (DIC) were used for observations, and digital images were taken with Axiocam and AxioVision (v. 3.1 software at 1300 × 1030 dpi) or Canon digital cameras.

Arbuscular mycorrhizal cultures

Bait cultures with soils from primary evergreen forest (Sierra del Rosario) were established in greenhouse at Ecology and Systematics Institute on *Sorghum bicolor* (L.) Moench and *Plantago major* L. as host plants (Sieverding 1991) in 1.0 L pots. An approximately 200 g field soil sample previously sieved by 2 mm mesh and including rootlets of native plants formed a sparse layer in the 1L plastic pot, which was previously filled to the midpoint with autoclaved primary evergreen forest soil: quartz sand mixture 3:1 (v:v). The pot was refilled with sterilized mixture and sown with seeds of the selected plants.

Voucher specimens were deposited in the herbaria of Instituto de Ecología y Sistemática, la Habana, Cuba (HAC) and Universidade Federal de Pernambuco, Recife, Brazil (URM).

Pure cultures were made by inoculating *Sorghum bicolor* and *Plantago major* seedlings germinated in autoclaved quartz sand with 15–20 spores of *G. crenatum* isolated from forest soils. 1L pots were filled with autoclaved primary evergreen forest soil: quartz sand mixture 3:1 (v:v) and watered to field capacity. A small hole was punched in the pot center using a glass shaker and the spores were discharged in the hole using Pasteur pipettes. Thereafter plant seeds were sown and the plantlets grew for 3–4 months in 10 pots.

Taxonomy

Glomus crenatum Furrazola, R.L. Ferrer, R.A. Herrera & B.T. Goto, sp. nov.

MycoBank MB 518874 Figs 1–9

Sporocarpia ignota. Sporae singulatim in solo vel rarum in radicibus efformatae, brunneae vel luteo-brunneae, globosae vel subglobosae, 100–205 µm diam. Tunica sporae stratis duobus: stratum exterior hyalinum, 0.5–2.0 µm crassum, secundum stratum, laminatum, 4.5–11.5 µm crassum, superficie exterioris colliculare, colliculis 5.5–18 µm altis, 7.5–28 µm diam ad basem. Strata sporarum continuantes stratis hypharum subtendarum; strata secundum hypharum subtendarum concolorata et continuantes cum stratae sporarum, hypha subtenda lobulata vel irregulariter ramosa, valde recta, acute recurvata, ad basem sporae 11–30 µm crassa, ad basem sporarum; hyphae 4.0–15 µm crassae pariete hypharum hyalinohyalina.

Type: Cuba, Pinar del Rio province, Reserva de la Biosfera "Sierra del Rosario", Loma El Salón, Vallecito Izquierda, primary evergreen forest on a convex hillside with N-NE exposition (400 to 425 m.a.s.l), 30 Nov.1994, R.A. Herrera, permanent slide mounted in PVLG (**Holotype**: HAC, **Isotype**: URM82278).

Etymology: *crenatum* (Latin), referring to the distinctive ornamentation on the outer surface of the glomerospore.

Glomerospores formed singly in soil and terminally on hypha (Fig. 1); yellow brown to orange brown when young (Figs 1–4), darkening slightly to dark brown when matured in soil (Figs 5–6); globose (100–208 µm diam.) to subglobose to elliptical (82–110 × 140–210 µm).

Spore wall 5.0–13.0 µm thick in total, consisting of two layers (SWL1, SWL2; Figs 2–3) that do not react in Melzer's reagent. Outer layer (SWL1) is

hyaline, semi-persistent, 0.5–2.0 µm thick, smooth in young spores (Figs 2–3), with granulations on the upper surface in mature spores. Inner layer (SWL2) is yellow brown to dark reddish brown, 4.5–11.5 µm thick (Figs 2–3), laminate, with hemispherical dome-shaped ornamentation, 7.5–28 µm diam., 5.5–18 µm tall (Figs 4–6). Distance between single projections are (5–)8–20(–40) µm. Ornamentation in planar view is circular to ellipsoid (Figs 5–6). Spore wall layers are continuous with the layers of the subtending hypha (Fig. 8).

Subtending hypha (sh) often detached at spore base; when present, single or occasionally double, straight to sharply curved, sinuous (Figs 7–9) or occasionally straight and parallel-sided, concolorous with wall SWL2, 11–30 µm wide (mean = 16.7 µm) at the point of attachment tapering to approx 2–6 µm at distance of 9 µm from the spore base; wall 4.0–15 µm thick (mean = 7.2 µm) near the spores base, tapering to approx. 1 µm distally; occlusion introverted spore wall thickening and by septum arising from SWL2 (Fig. 9). Pore at the subtending hypha sometimes partially open (Fig. 8). Subtending hypha convolutions often clump a lot of organic and soil particles or small pieces of roots. In PVLG-Cotton Blue, only SWL2 show a slight cyanophilous reaction in young hyphae.

Additional specimens examined: **CUBA. holguin province**, Moa-Sagua-Baracoa, from plantation of *Pinus cubensis* Griseb.; AMF hosts (e.g., *Cecropia* spp.) and other indigenous plants also present.

Germination is directly by regrowth of the subtending hypha.

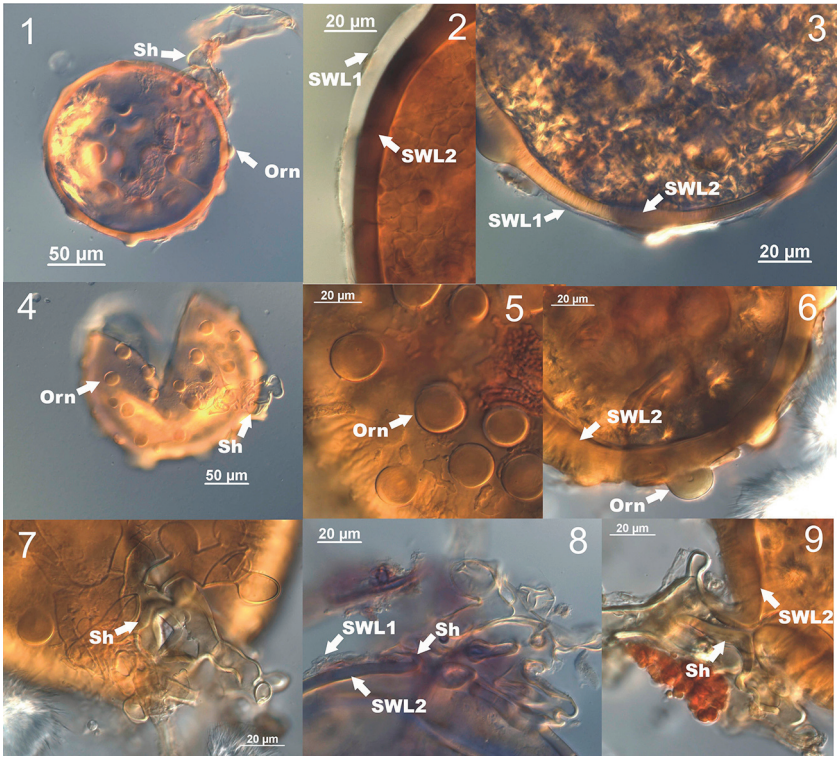
Arbuscular mycorrhiza formation is unknown to date.

Distribution — So far, the new fungus has been detected only in Cuba. Known in soil and litter of evergreen forests consisting mainly of *Pseudolmedia spuria*, *Oxandra lanceolata*, *Trophis racemosa*, *Matayba apetala*, *Dendropanax arboreus*, and *Calophyllum antillanum* in native, ecologically stable (climax) tropical evergreen forest ecosystems in Sierra del Rosario, Pinar del Rio (Cuba occidental).

Discussion

Glomus crenatum is readily distinguished from previously described *Glomus* species by its ornamented spores covered with collicular outgrowths. *Glomus pustulatum* Koske et al. 1986 also produces spores with collicular ornamentation, but its spores are paler, smaller (40–140 x 60–140 µm), and with a three-layered spore wall (vs. two layers in *G. crenatum*) (Koske et al 1986). Additionally, the ornamentation in *G. pustulatum* is a part of the outermost spore wall component (SWL1), whereas in *G. crenatum* the collicular outgrowths cover the inner structural laminate spore wall layer (SWL2).

Glomus multicaule Gerd. & B.K. Bakshi 1976, which also forms ornamented, darkly pigmented glomerospores with projections on the laminate layer SWL2,



FIGS. 1–9. *Glomus crenatum*. **1**. General aspect of glomerospores in PVLG; note the colliculate ornamentation (Orn) on the spore wall (SW). **2–3**. Spore wall layers (SWL1 and SWL2); micrographs taken using Nomarski interference. **4–6**. Dome shaped, hemispheric, ornamentation (Orn) on spore wall. **7–8**. Irregularly branched and/or convoluted subtending hyphae (sh); note the open pore in the subtending hypha. **9**. Septum formed by SWL2.

differs from *G. crenatum* by having two or more attachment hyphae, very irregular spores (149–249 × 124–162 µm), and small projections that are only 1.2–3.7 µm high (Gerdemann & Bakshi 1976).

The irregularly convoluted subtending hyphae found in *G. crenatum* (FIGS 7–9) resemble those described for *G. fuegianum* (Speg.) Trappe & Gerd. 1974 (Gerdemann & Trappe 1974) and *G. badium* Oehl et al. 2005 (Oehl et al. 2005), both of which are known to form dense sporocarps in soil. Such structures are generally difficult to observe due to adherent soil and organic matter particles (FIG. 9) or because they become detached close to the spore base. However, such structures suggest that *G. crenatum* might also form compact sporocarps in soil. However, spore aggregations have not yet been observed in the Cuban tropical forest soils investigated.

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